Nov. 3, 1953 E. R. HARMS 2,658,077

CRYSTALLIZATION OF CHLORTETRACYCLINE

Filed Aug. 20, 1949 4 Sheets-Sheet 1

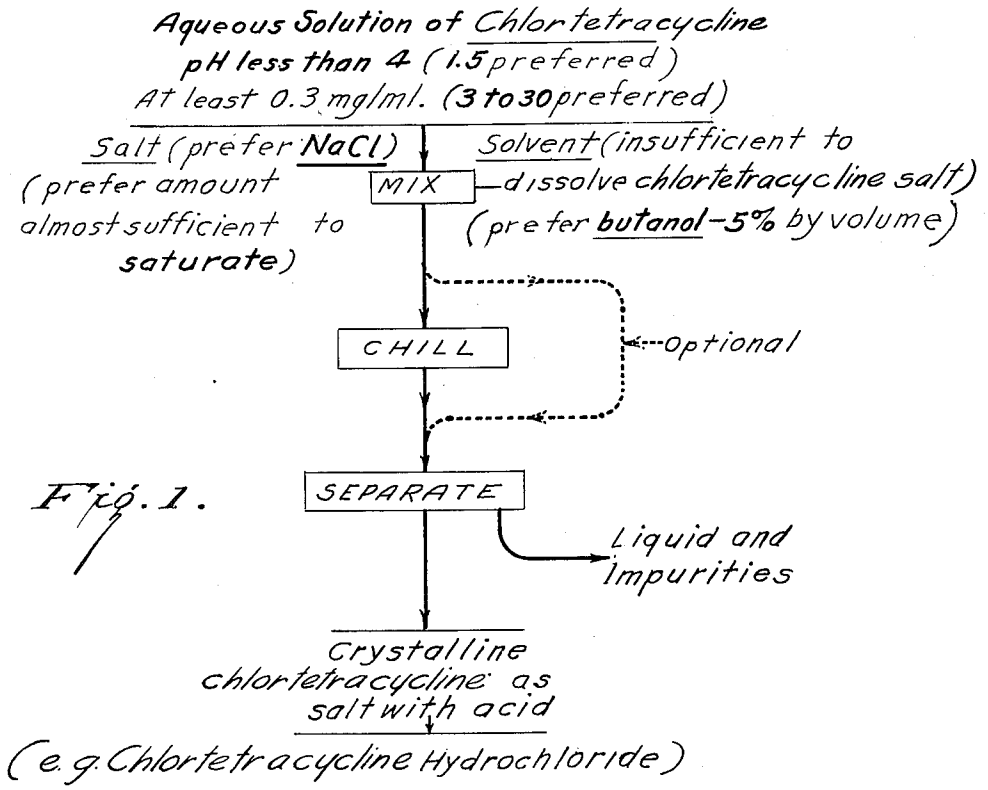


Fig. 1.

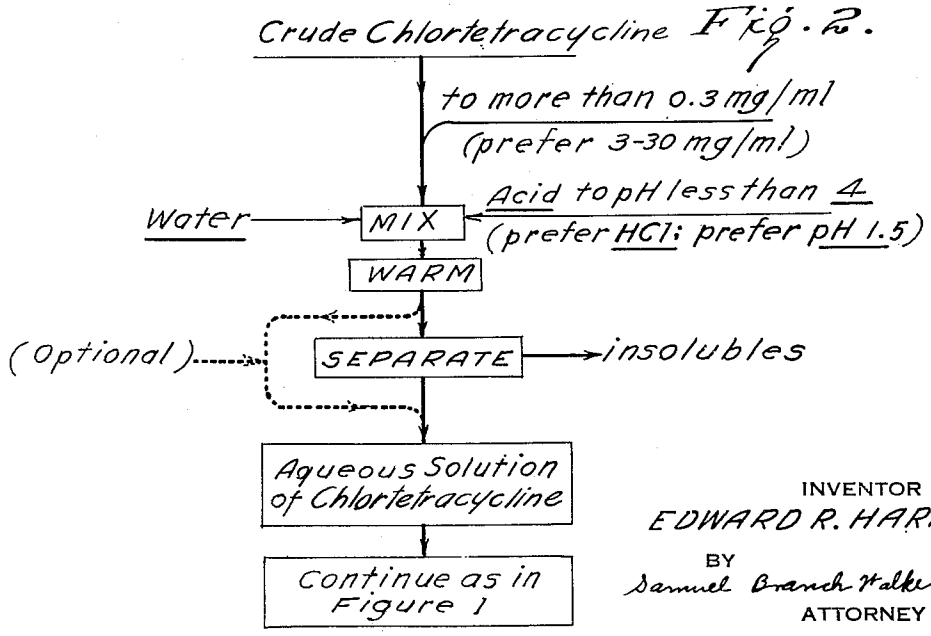


Fig. 2.

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CRYSTALLIZATION OF CHLORTETRACYCLINE

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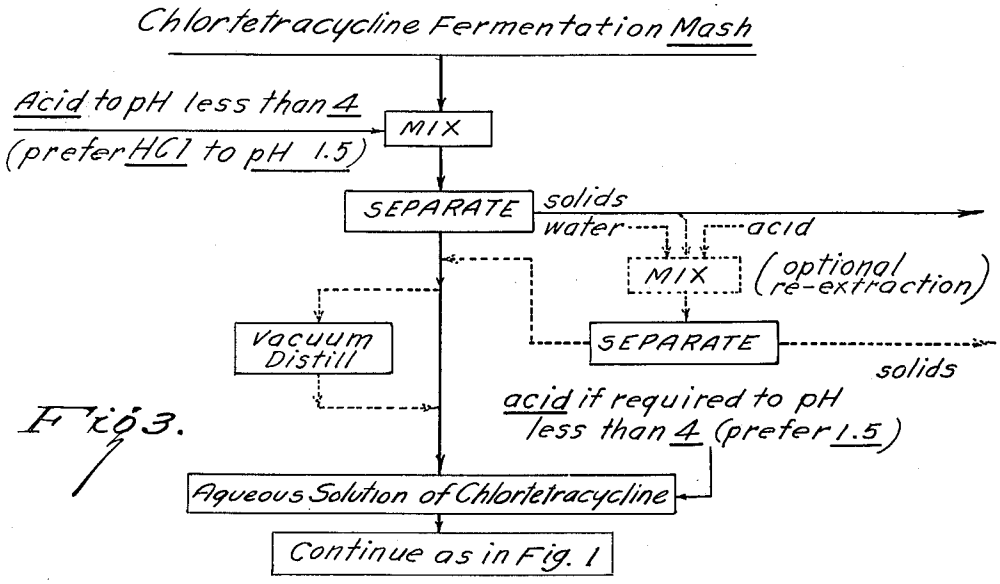


Fig. 3.

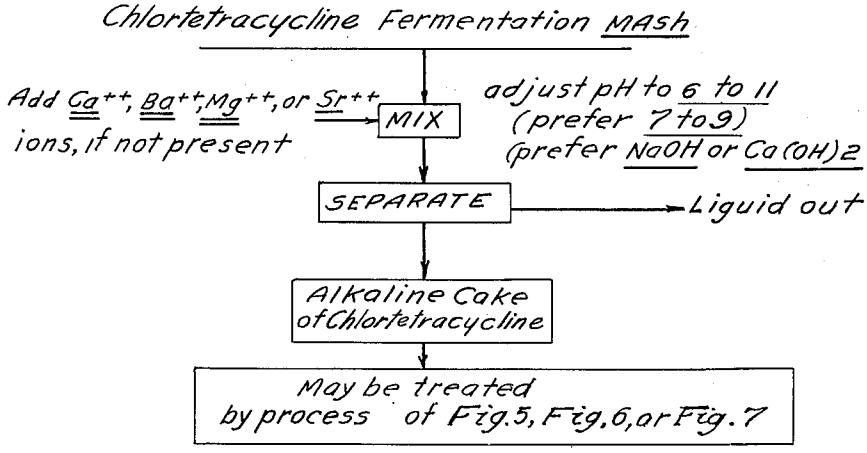
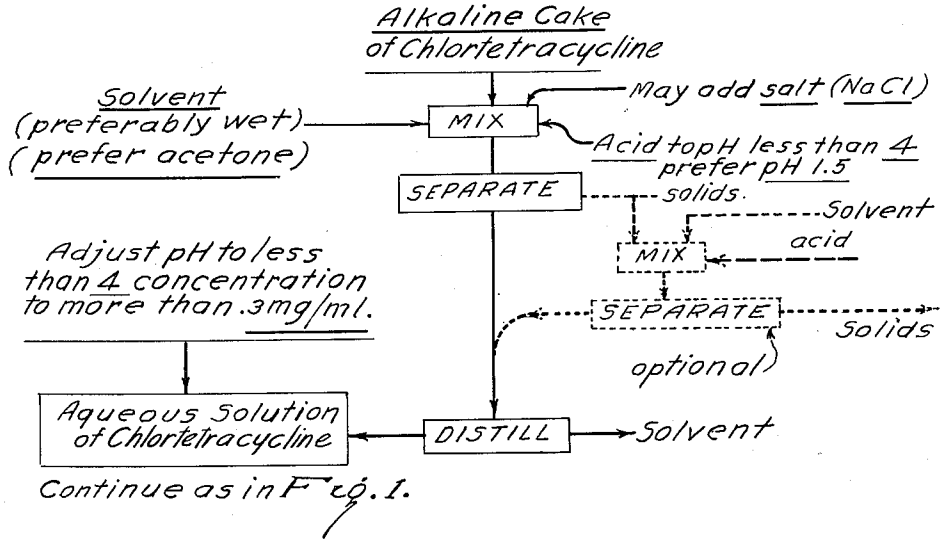


Fig. 4.

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Fig. 5.
Acid-solvent Extraction



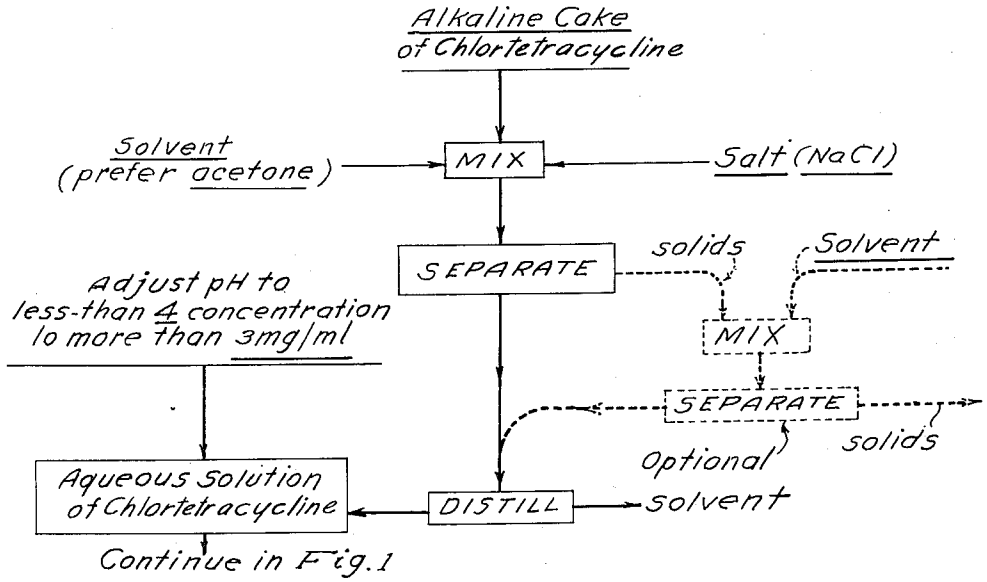


Fig. 6.
Alkaline-Solvent Extraction

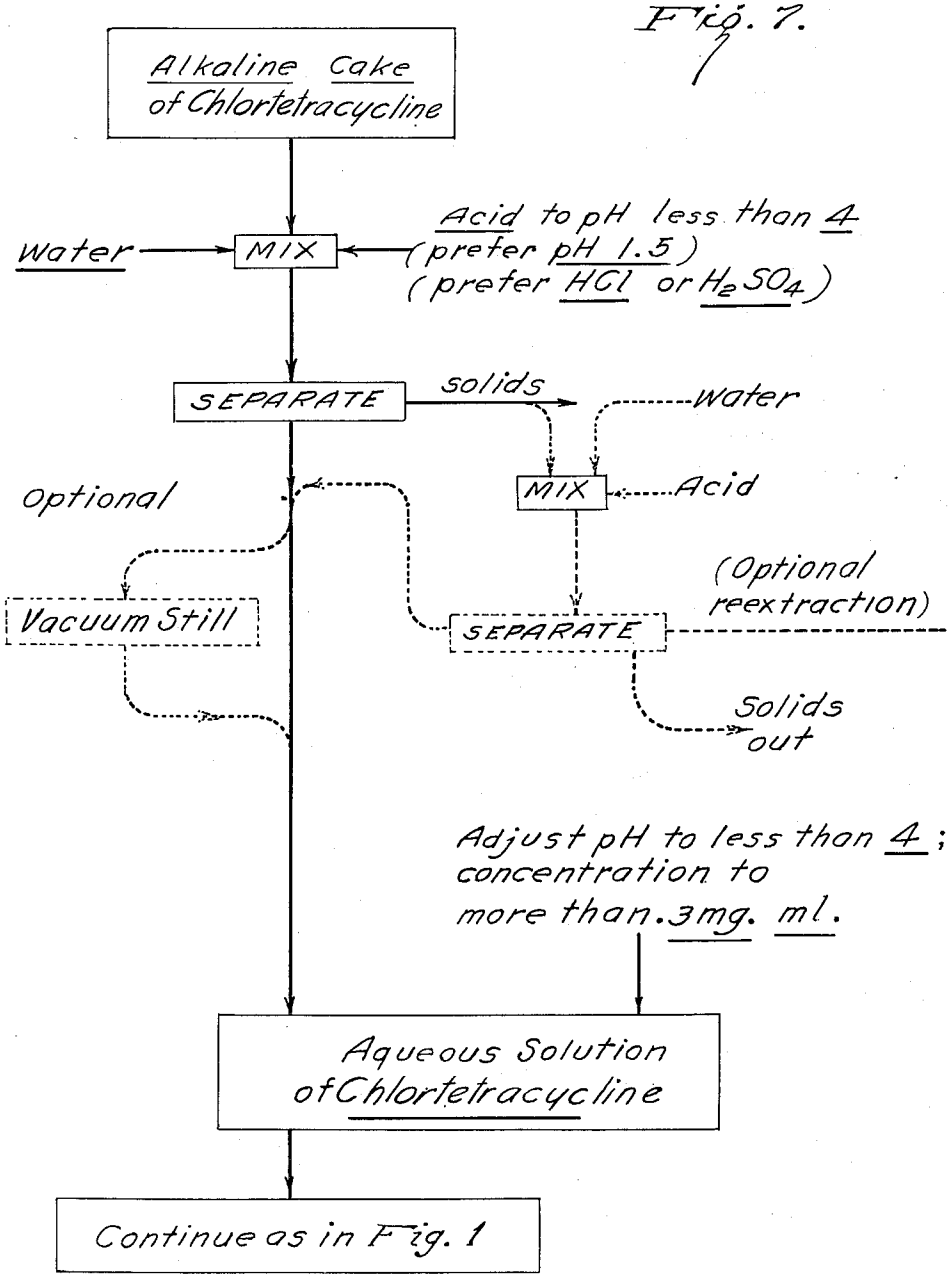
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CRYSTALLIZATION OF CHLORTETRACYCLINE

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Fig. 7.



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CRYSTALLIZATION OF CHLORTETRACYCLINE

Edward R. Harms, Park Ridge, N. J., assignor to American Cyanamid Company, New York, N. Y., a corporation of Maine

Application August 20, 1949, Serial No. 111,581

6 Claims. (Cl. 260—559)

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This invention relates to a method for the isolation of chlortetracycline from the media from which it is produced, or a solution in which it is present, and the purification of the chlortetracycline. It has for its object a distinctive and novel process whereby the chlortetracycline may be most conveniently recovered from an aqueous solution and has as a further object the transformation of the chlortetracycline to a therapeutically desirable form. The organism *Streptomyces aureofaciens* during its growth produces a substance known as chlortetracycline which has peculiar and remarkable activity as an antibiotic having a greater therapeutic range than any of the previously known antibiotics. The antibiotic and certain methods of fermentation, and certain uses therefor are set forth in an application of Benjamin M. Duggar, Serial Number 7,592, filed February 11, 1948, now Patent 2,482,055, September 13, 1949. Additional information on both the organism and the properties of chlortetracycline are disclosed in the Annals of the New York Academy of Sciences, volume 51, article 2, November 1948; and much of the current medical literature contains references to remarkable results being presently attained with this potent antibiotic.

In the accompanying drawings, Figure 1 shows diagrammatically the method of obtaining purified chlortetracycline from an acidified aqueous solution containing chlortetracycline.

Figure 2 shows the preparation of the acidified aqueous solution of chlortetracycline from a crude chlortetracycline.

Figure 3 shows the preparation of the acidified aqueous solution directly by acidifying a fermentation mash.

Figure 4 shows the preparation of an alkaline cake of chlortetracycline from the fermentation mash.

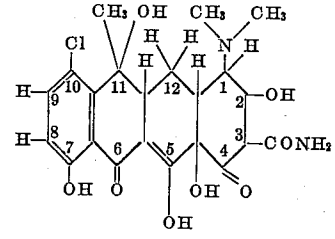
Figure 5 shows the preparation of the acidified aqueous solution by extracting the alkaline cake with solvent and an acid, followed by removal of the solvent.

Figure 6 shows the preparation of the acidified aqueous solution by extracting the alkaline cake with a solvent, while still alkaline, and removal of the solvent and acidification.

Figure 7 shows the preparation of the acidified aqueous solution by extraction of the alkaline cake with acid water.

Chlortetracycline is produced and sold under the commercial designation "Aureomycin" which, in certain countries, is a trade-mark of the American Cyanamid Company. Chlortetracycline has been found to be a naphthacene derivative and while having a structure which strongly suggests tautomeric forms, is believed to exist with the following probable structure:

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A numbering system is shown for the molecule. One proper name for the compound is 1-dimethylamino-4, 6-dioxo-10-chloro - 11 - methyl-2,4a,5,7,11 - pentahydroxy - 1,4,4a,6,11,11a,12,12a-octahydronaphthacene - 3 - carboxamide. The generic term for the compound is chlortetracycline, sometimes spelled "chlorotetracycline." (Note: Journal of the American Chemical Society, 74: 4976 (1952)).

Whereas in the past chromatographic adsorption and other methods have been used for the isolation of chlortetracycline, including steps of salting out of chlortetracycline into solvents, etc., I have discovered that the chlortetracycline may be particularly conveniently separated from an acid aqueous media by the addition of a solvent which may or may not be miscible with the water which is added in sufficient quantity to induce crystallization of the chlortetracycline from the aqueous phase, but which is not added in sufficient quantity to dissolve or extract the chlortetracycline. Frequently n-butanol is used. Preferably a salt such as sodium chloride is added to the aqueous layer for the several purposes of assisting in causing the chlortetracycline to come out of the aqueous layer, to insure a better break between the water and the solvent, and to cause the chlortetracycline to come out as the particular acid salt which is desired, frequently the hydrochloride. As steps in the preparation of the aqueous chlortetracycline containing material, the chlortetracycline may be concentrated from a more dilute acid aqueous solution by vacuum distillation. Alternatively the chlortetracycline containing material may be caused to go into an aqueous layer by extraction of the chlortetracycline from a more dilute solution or from solids, such as the filter cake, by the use of a suitable solvent such as acetone; which is conveniently wet and serves as a source of water for the residual aqueous chlortetracycline-con-

taining layer after the acetone is removed by distillation, preferably at a comparatively low temperature. A salt such as sodium chloride assists in securing an efficient separation. As an alternative the cake may be dried and extracted with an anhydrous solvent, and water later added for the preparation of the comparatively concentrated acid aqueous chlortetracycline-containing liquor.

Certain steps in the refining of chlortetracycline herein disclosed are claimed in a copending application of Edward E. Starbird and Charles Pidacks, Serial No. 364,182, entitled Chlortetracycline, Purification and Alkaline Earth Salts, filed June 25, 1953, which is a continuation-in-part of applications Serial Numbers 62,722 and 62,766 filed November 30, 1948, now abandoned in favor of said Serial Number 364,182.

The aqueous chlortetracycline-containing material may be the liquor resulting from any of several preceding steps but most conveniently is either the fermentation mash itself or the liquid extract obtained by filtering the fermentation mash in the presence of an alkaline material containing ions selected from the group consisting of calcium, barium, magnesium and strontium ions, at a pH of from 6 to 11, the best fractionation being from 7 to 9, separating the resulting solids containing an insoluble salt of chlortetracycline and extracting the solids with an acid and using this resultant acid extract. Of course, a repeated or counter-current extraction of the solids tends to give an improved yield. The acid solution containing the chlortetracycline is most efficiently handled at a pH of less than about 4. If the pH is much higher than 4, the solubility characteristics are not as favorable; and for normal operations, it is preferred that the pH be not far below 1. A lower pH is theoretically satisfactory but in plant processes the corrosion induced by such an acidic solution causes corrosion problems or requires the use of remarkably acid resistant vessels, with no resulting compensating economic advantage. It is most convenient to use a pH of in the neighborhood of 1 to 2, and a pH of 1.5 is normally preferred as a reasonable compromise, economically, of all of the factors involved including corrosion, the cost of the acid, solubilities, and recovery, although with some equipment corrosion factors may necessitate the treatment at a pH of 3. A solvent extraction may be used, such as acetone, or other ketone, ethanol, or other alcohol, and the chlortetracycline transferred to an acid aqueous solution by evaporation of the solvent, or other suitable method.

The aqueous solution then containing the chlortetracycline may be concentrated by vacuum distillation if not already sufficiently concentrated. Some decomposition results from a temperature in excess of about 50° C. With normal plant conditions, a temperature in the range of 20 to 40° C. is preferred as a compromise between the cost of cooling the condenser and the losses at the higher temperatures. The water may even be removed from the frozen phase, after cooling below freezing, but such drastic conditions are not necessary. The distillation of a solvent, if present, and the water from the aqueous phase is continued until a suitable concentration of chlortetracycline in the residual aqueous phase is obtained. The degree of concentration depends upon purities and various other factors. Chlortetracycline is soluble in the aqueous phase to the extent of about 10 to 15 milligrams per milliliter at a pH of 1.5 at room temperature. It is preferred that the concentration be continued until the concentration of chlortetracycline in the aqueous phase is from 3 to 30 milligrams per milliliter, depending upon the impurities present. The concentration should be at least 0.3 milligram per milliliter for reasonable separation when salt is added. With certain chlortetracycline mashes the preferred concentration may be obtained by concentrating the aqueous phase to approximately 25% or less of the original mash volume. Upon cooling and agitation the chlortetracycline will not readily separate from the aqueous phase. It may however, if permitted to stand, separate as a comparatively impure product in an amorphous state. If however, in accordance with this invention a small quantity of a solvent and a salt is added, the material will tend to separate in a comparatively pure crystalline form. Best results are obtained if the quantity of salt is such that the solution will be nearly saturated. For the purposes of my invention I prefer the use of sodium chloride as the salt because therefrom results chlortetracycline as the hydrochloride, and sodium chloride is cheap.

Other salts may be used. Potassium chloride and ammonium chloride give a nicely crystallized chlortetracycline hydrochloride. With calcium and lithium chlorides the chlortetracycline has more of a tendency to remain in the amorphous state or separate as an oil; but these too may be used. The salts of other acids such as sodium sulfate, ammonium sulfate, lithium sulfate, disodium phosphate, mono-potassium phosphate, etc. may be used but the products are more difficult to crystallize; and from the therapeutic standpoint the medical profession appears to prefer the hydrochloride, and accordingly, chlorides are the salts which are normally to be desired. Any amount of salt, less than a completely saturated solution, improves the separation of the chlortetracycline hydrochloride. Usually the more salt, the better yields. Slightly less than saturated solutions are convenient, as they give good yields, and do not themselves tend to precipitate the salt itself. The solvent present has several purposes. It tends to dissolve certain of the solvent soluble impurities and remove them from the crystals. It tends to cause the separation to occur more readily, particularly if it furnishes a solvent-aqueous interface, and it assists in the formation of the desired crystalline modification of chlortetracycline. Some several solvents may be used.

Among the more useful solvents are the middle chain alcohols. The lower alcohols tend to be more soluble in water than is desirable. Methyl, ethyl and isopropyl alcohols cause separation of the chlortetracycline in the crystalline form with salt but do not cause two distinct phases at the usual temperature and concentrations. Such solvents as normal butyl alcohol, tertiary butyl alcohol, secondary butyl alcohol, amyl alcohol, hexyl alcohol, octyl alcohol, capryl alcohol, decanol, etc. all form two phases and permit the separation of the crystalline form of chlortetracycline at a liquid-solvent interface, and as such give a more conveniently utilized modification of the invention. The alcohols above decanol tend to become more viscous and

harder to handle. The alkoxy ethanols and other polyhydric alcohols and their esters are normally also satisfactory. Butanol is a preferred solvent because it is readily obtainable commercially, at a comparatively low price, is not so soluble in water as to cause difficulties in phase separation, and does not dissolve a disproportionately large quantity of the chlortetracycline.

Just sufficient butanol to form a separate phase gives the improved crystallization effect but if about 5% of normal butanol is added the butanol dissolves a larger proportion of the impurities without dissolving an unreasonably large proportion of the chlortetracycline. If more than 10% butanol is added the loss of product in the solvent usually becomes disproportionately great.

The extraction is conveniently carried out at room temperature and the resulting crystalline chlortetracycline filtered from the two liquid phases. It may be washed with a solvent, or several solvents, and dried. Ethanol and water are cheap and convenient; other alcohols or ketones etc. may be used. The product obtained under my preferred conditions is a crystalline chlortetracycline, the purity of which depends somewhat upon the starting material. If other than the preferred procedures are used, additional purification may become necessary. An additional recrystallization usually improves the purity even though it may not be necessary.

The following specific examples are intended to show certain embodiments of the invention but it is to be understood that it is impractical to show all modifications which will work or to show each of the combinations of solvents in conjunction with each of the salts, etc. There are many flexible steps in the procedure in which a slight variation at one point may be compensated by or may require an additional modification at another point to obtain the same result as would be secured by closely following the procedures as set forth, all of such modifications being of course within the scope of this invention as set forth in the appended claims.

EXAMPLE 1

Direct acidification

To 4.9 liters of fermented mash containing chlortetracycline was added 135 milliliters of 6 normal hydrochloric acid which lowered the pH to 1.45. The mixture was agitated to insure thorough mixing, and solution of all the chlortetracycline. To an aliquot of 3000 milliliters of the resulting solution was added 300 grams of the filter-aid known as Hy-Flo Supercel and the material filtered through paper, and then washed with sufficient acidulated water that 3000 milliliters of filtrate were obtained. The filtrate was placed in a vacuum still and distilled within the temperature range of 25 to 30° C. under vacuum until a 550 milliliter concentrate was obtained. 50 milliliters were retained for assay samples and the remaining 500 milliliters had added thereto 25 milliliters of normal butyl alcohol and 125 grams of sodium chloride. The mixture was agitated at room temperature for one-half hour, then stored overnight at 2° C. The crystals were removed by a Buchner funnel, washed once with a small quantity of alcohol, then with distilled water, and the thus isolated crystals were vacuum dried. There was obtained a yield of 4.61 grams analyzing 300 gammas per milligram by a colorometric assay which is a recovery of 37% from the total mash to the crystals.

EXAMPLE 2

Alkali-acid-process

To 30 liters of chlortetracycline mash resulting directly from the fermentation tanks, at a pH of 6.3 was added 125 milliliters of a 10% slurry of calcium hydroxide and 300 grams of the filter-aid known as Hy-Flo Supercel. The material was agitated at room temperature for 10 minutes and the resulting material at a pH of 8.8 was filtered, and the cake washed in 3 liters of water. To 6 liters of water was added 250 milliliters of a 1 to 4 mixture of sulfuric acid and the acidified water heated to 50° C. and the filter cake prepared above added. The pH which was then approximately 4.0 was lowered to a pH of 1.35 by adding additional 1 to 4 sulfuric acid, approximately 250 milliliters being required. The mixture was agitated at a temperature of between 45 and 50° C. for 10 minutes and filtered. The residual cake was additionally extracted by a second 6 liters of water having added thereto sufficient sulfuric acid to keep the pH at 1.45, the cake being stirred for 10 minutes and the material refiltered. The cake was discarded and the extracts found to measure 15.5 liters. An aliquot of 10 liters of the cooled extracts were vacuum distilled in a glass and stainless steel still at a temperature between 30 and 36° C. to 2600 milliliters. To the thus prepared concentrate, now at a pH of 1, was added 150 milliliters of normal butanol and 780 grams of sodium chloride. The mixture was agitated for 30 minutes and held in a cold room between 2–5° C. overnight. The crystals which formed were washed with ethanol, then twice with water, then again with ethanol until the washing alcohol was light in color; the total volume of water washings being 110 milliliters and the total volume of alcohol washings being 120 milliliters. There was obtained a crystal weight of 10.8 grams of chlortetracycline hydrochloride with a crystal potency of 970 gammas per milligram. This is a remarkably pure chlortetracycline hydrochloride to be thus simply obtained.

EXAMPLE 3

Alkali-acid, acetone process

30 liters of fermentation mash had added thereto 10 normal sodium hydroxide until the pH was approximately 9; 50 milliliters being required. 500 grams of Hy-Flo Supercel filter-aid were added and the solids separated by filtration. To the cake was added 4.5 liters of 90% acetone (10% water) and thereto added sufficient 1 to 4 sulfuric acid to lower the pH to 1.4; approximately 450 milliliters being required. To the mixture was added 700 grams of sodium chloride, the mixture agitated for 15 minutes, then filtered. 7 liters of filtrate were obtained. The cake was re-extracted with 4.5 liters of 90% acetone, 90 milliliters of sulfuric acid being required to keep the pH at 1.4, and 350 grams of sodium chloride being added. The slurry was agitated for 15 minutes and filtered; 3.8 liters of filtrate being obtained. The cake was washed with 3 liters of acetone and blown dry. The pooled acetone extracts were vacuum concentrated to 4150 milliliters, all of the residual being water, and adjusted to a pH of 2.5; 33.5 milliliters of sulfuric acid being required. The material was filtered, and to the clear filtrate were added 207 milliliters

of butanol and 1245 grams of sodium chloride. The mixture was agitated for one hour, then filtered on a Buchner funnel, washed with alcohol, water, and again with alcohol. There was obtained a yield of 13.5 grams of chlortetracycline hydrochloride analyzing 1000 gammas per milligram; this amounting to a recovery of 48% of the mash activity. The product as thus obtained is satisfactory for therapeutic purposes.

EXAMPLE 4

Alkaline acetone process

8,150 liters of fermented chlortetracycline mash was adjusted to a pH of 8.5 with 10 normal sodium hydroxide. The material was filtered, and the filtrate was discarded. The cake was washed with a small volume of water, then slurried at room temperature with 1200 liters of acetone. The mixture was adjusted to a pH of 10 with 10 normal sodium hydroxide and after 15 minutes' agitation, 400 kilograms of sodium chloride was added, and the mixture stirred for an additional 15 minutes, and then filtered. The cake was again extracted with an additional 800 liters of acetone, the mixture being adjusted to a pH of 10.2 with 10 normal sodium hydroxide, and agitated for 15 minutes, and the acetone removed by filtration. The pooled extracts now measuring 1,498 liters, were concentrated by vacuum distillation at a temperature between 25 and 35° C. until all of the acetone was removed. The concentrate is about 10% by volume of the original volume of the acetone. The aqueous concentrate was adjusted to a pH of 1.5 with 4 molar sulfuric acid, 5% of its volume of butanol added, the mixture stirred and permitted to stand overnight. The crystals were removed, washed with ethanol, water, and again with ethanol, and then dried.

EXAMPLE 5

Alkali acid process

A fermented chlortetracycline mash was adjusted to a pH of 8.5 with a 10% slurry of calcium hydroxide and the mash filtered. The filter cake was added to a volume of water equivalent to 20% of the original mash volume at a temperature of 50° C. and enough hydrochloric acid added to lower the pH to between 3 and 4. After the cake was thoroughly slurried, the pH was adjusted to 1.5 and the slurry agitated for an additional 10 minutes. The slurry was filtered and the cake extracted a second time with a similar volume of water, the water extracts combined and concentrated at a temperature of between 30 and 40° C. to 25% of their original volume. To the concentrate was added 5% by volume of normal butanol and 30% weight/volume sodium chloride added and stirred for 90 minutes. The crystals thereby formed were separated, washed with ethanol, water, and again with ethanol and vacuum dried. The chlortetracycline hydrochloride as obtained was satisfactory for therapeutic purposes.

EXAMPLE 6

A fermented chlortetracycline mash was adjusted to a pH of 8.5 with a 10% slurry of calcium hydroxide, and the mash filtered. The filter cake was added to a volume of water equivalent to 20% of the original mash volume and enough hydrochloric acid added to lower the pH to 4. The cake was thoroughly mixed and the slurry filtered. The extract was concentrated by vacuum distillation at a temperature of not more than 50° C. to approximately 20% of its original volume. To this concentrate was added 5% by volume of normal butanol and 30% by weight of sodium chloride. The mixture was thoroughly stirred and the crystals thereby formed separated, washed with ethanol, and vacuum dried. Chlortetracycline hydrochloride was thereby recovered.

EXAMPLES 7–18

A fermented mash was adjusted to a pH of 8.5 with 10 normal sodium hydroxide and the mash filtered. The filter cake was added to a volume of water equivalent to 20% of the original mash volume, and enough hydrochloric acid added to lower the pH to approximately 1.5, and the resulting mixture again filtered. The extract was concentrated by vacuum distillation at a temperature between 30 and 40° C. to 25% of its original volume. To the concentrate was added 30 parts by weight of sodium chloride per 100 parts by volume of liquid. The solution was separated into a number of aliquot parts and 5% by volume of various solvents were added thereto. In each instance crystals of chlortetracycline hydrochloride separated and were recoverable on a Buchner funnel.

Example No.	Solvent	Appearance	Result
7	Anhydrous ethanol.	1-phase	Crystals.
8	Methanol	do	Do.
9	Isopropanol	do	Do.
10	n-Butanol	2-phase	Crystals at interface.
11	Tert. butanol	do	Do.
12	Sec. butanol	do	Do.
13	Amyl alcohol	do	Do.
14	Hexyl alcohol	do	Do.
15	Octyl alcohol	do	Do.
16	Capryl alcohol	do	Do.
17	Decanol	do	Do.
18	2-ethyoxy ethanol	1-phase	Crystals.

In each instance the crystals as initially separated were a medium dark brown but upon washing with ethanol, water, and ethanol, became a lighter yellow color.

EXAMPLES 19–28

To aliquot portions of the concentrated filtrate containing the chlortetracycline in liquid form were added a series of salts so that the liquid phase was nearly saturated with each, the quantity added varying with the solubility of the respective salts, and to the salt-containing liquor was added in each instance 5% by volume of butanol. The material was cooled, permitted to stand with shaking and in each instance the chlortetracycline separated out.

Example No.	Salt	Form of chlortetracycline recovered
19	Sodium chloride	Crystals—hydrochloride salt.
20	Ammonium chloride	Do.
21	Lithium chloride	Oil—hydrochloride salt.
22	Potassium chloride	Crystals—hydrochloride salt.
23	Calcium chloride	Amorphous—hydrochloride salt.
24	Sodium sulfate	Amorphous sulfate salt.
25	Ammonium sulfate	Do.
26	Lithium sulfate	Do.
27	Di-sodium hydrogen phosphate.	Amorphous phosphate salt.
28	Potassium di-hydrogen phosphate.	Oily phosphate salt.

The chlortetracycline obtained in the oily or amorphous form could be separated and converted to a crystalline hydrochloride by treating it with a small portion of anhydrous ethanol and concentrated hydrochloric acid.

EXAMPLE 29

High solvent

To an aliquot obtained as in the preceding series of examples was added 30 parts by weight of sodium chloride to 100 parts by volume and 10% by volume of normal butanol. After standing in a chill chamber for 24 hours, the crystals were filtered off. The larger volume of butanol dissolved more of the impurities so that the chlortetracycline hydrochloride obtained appeared to be in a slightly purer form; but the yield was lessened because of the amount dissolved by butanol.

EXAMPLE 30

One liter of fermentation mash was raised to a pH of approximately 9 by the addition of 10 normal sodium hydroxide, 25 grams of a diatomaceous earth filter-aid were added, and the solids separated by filtration. The cake, after washing, was added to 250 milliliters of water acidified to a pH of 1.35 with sulfuric acid, and after stirring, additional sulfuric acid was added to keep the pH at 1.35. The slurry was filtered, and to the acidified extract was added 5% by volume of butanol and 25% weight per volume of sodium chloride. The mixture was stirred and chilled, and the resulting crystals of chlortetracycline hydrochloride separated in a Buchner funnel, and washed with water. Crystalline chlortetracycline hydrochloride was thereby recovered.

EXAMPLE 31

To 5 liters of fermented mash containing chlortetracycline was added sufficient 6 normal hydrochloric acid to acidify the material to a pH of 1.4. The mixture was thoroughly agitated, then filtered. To the filtrate was added 5% by volume of normal butyl alcohol and 30% weight per volume of sodium chloride. The mixture was agitated at room temperature for one hour, then stored overnight at 2° C. The crystals were removed by a Buchner funnel and washed as in Example 1. Thereby was obtained crystalline chlortetracycline hydrochloride.

EXAMPLE 32

15 grams of an impure chlortetracycline hydrochloride were suspended in 1 liter of distilled water, and thereto added sufficient hydrochloric acid to lower the pH to 1.0. The mixture was warmed to about 50° C. and stirred for 30 minutes to insure complete solution of all of the chlortetracycline present. Insolubles were removed by filtration, 250 grams of sodium chloride were added in 50 milliliters of normal butanol. The mixture was cooled with stirring to 2° C. and permitted to stand overnight in a chill room. The crystals thereby formed were separated on a Buchner funnel and washed with ethanol, water, again with ethanol, and then dried. The recovered chlortetracycline hydrochloride thus formed was found to be much purer than the starting material.

The above set forth examples show certain preferred examples and some of their modifications whereby the chlortetracycline may be recovered from the fermentation liquor. Obviously, to those skilled in the art, the variations within the scope of the appended claims will suggest themselves, and certain variations in concentrations, temperatures and conditions will be necessitated by minor variations in the strains, purities and other operating conditions. It is of course possible to operate at higher temperatures, greater concentrations or lesser concentrations, etc. with, of course, compensating variations in the yield.

Having thus described and set forth certain aspects thereof in detail, as my invention I claim:

1. A method of isolating chlortetracycline hydrochloride which comprises forming an acid solution at a pH of less than 4 containing from 3 to 30 milligrams of chlortetracycline per milliliter, adding 25 to 30% of sodium chloride and not more than 10% normal butanol, and separating the thus formed chlortetracycline hydrochloride from the combined aqueous and butanol liquid present.

2. A method of isolating chlortetracycline as the hydrochloride salt which comprises forming an acid solution containing chlortetracycline at a pH of between approximately 1 and 2, adding thereto from 25 to 30 parts by weight of sodium chloride per 100 parts by volume of solution and approximately 5% of normal butanol, agitating and chilling the mixture and recovering the thus formed chlortetracycline hydrochloride salt from the combined aqueous and butanol liquid present.

3. A method of isolating chlortetracycline which comprises adding a salt of the group consisting of sodium chloride, ammonium chloride, lithium chloride, potassium chloride, calcium chloride, sodium sulfate, ammonium sulfate, lithium sulfate, di-sodium hydrogen phosphate and potassium di-hydrogen phosphate, and a solvent in a quantity insufficient to dissolve the chlortetracycline present from the group consisting of ethanol, methanol, isopropanol, n-butanol, tertiary butanol, secondary butanol, amyl alcohol, hexyl alcohol, octyl alcohol, capryl alcohol, decanol and 2-ethoxy ethanol to an aqueous solution containing chlortetracycline at a pH of less than 4 at a concentration of at least .3 milligram per milliliter and separating and recovering the thus formed acid salt of chlortetracycline from the combined aqueous and solvent liquid present.

4. A method of isolating chlortetracycline as an acid salt which comprises adding a salt from the group consisting of sodium chloride, ammonium chloride, lithium chloride, potassium chloride, calcium chloride, sodium sulfate, ammonium sulfate, lithium sulfate, di-sodium hydrogen phosphate and potassium di-hydrogen phosphate, and an organic solvent in a quantity insufficient to dissolve the chlortetracycline present from the group consisting of ethanol, methanol, isopropanol, n-butanol, tertiary butanol, secondary butanol, amyl alcohol, hexyl alcohol, octyl alcohol, capryl alcohol, decanol and 2-ethoxy ethanol to an aqueous solution containing chlortetracycline at a pH of less than 4 and at a concentration of from 3 to 30 milligrams per milliliter until the solution is substantially saturated with the salt and separating and recovering the thus formed acid salt of chlortetracycline from the combined aqueous and solvent liquid present.

5. A method of isolating chlortetracycline as the hydrochloride salt which comprises forming an acid solution containing at least about 3 milligrams per milliliter of chlortetracycline, adding thereto sufficient sodium chloride to substantially saturate the aqueous layer and approximately 5% by volume of normal butanol, and separating the thus formed chlortetracycline hydrochloride from the combined aqueous and butanol liquid present, all at a pH of less than about 4.

6. A method of isolating chlortetracycline as an acid salt which comprises adding an inorganic water-soluble salt of a mineral acid and an at least partially water-soluble hydroxylated organic solvent in a quantity insufficient to dissolve the chlortetracycline present to an aqueous solution containing chlortetracycline at a pH of less than 4 and a concentration of approximately 3 to 30 milligrams per milliliter, and separating and recovering the thus formed salt of chlortetracycline, with an acid, said acid being the acid derived from the anionic portion of said inorganic water-soluble salt.

EDWARD R. HARMS.

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Number	Name	Date
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2,482,055	Duggar	Sept. 13, 1949

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